Dispatches

Hedgehog Signalling: Kif7 Is Not That Fishy After All

Recent reports examining the mammalian kinesin relative Kif7 highlight the conserved role for microtubule motor proteins in *Drosophila* and vertebrate Hedgehog signalling. Mammalian Kif7 action centres at the primary cilium, an organelle absent from *Drosophila*. These studies raise interesting questions about the coupling of microtubule trafficking to the Hedgehog pathway.

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Our understanding of signalling pathways has reached a mature phase: molecular interactions are generally well understood and signalling components are largely conserved between species. However, regarding evolutionary conservation, studies of the Hedgehog (Hh) signalling pathway have tended to enlighten and confound in equal measure. Though Hh signals play a central role in patterning *Drosophila* and vertebrate embryos, comparisons have suggested a number of striking mechanistic differences. In some cases, the evidence for evolutionary divergence is compelling; for example, the primary cilium is central to Hh signalling in mammals but not in flies [1]. In other instances, the evidence is more contentious — a case in point being the apparent divergence in the function of the closely related kinesin family members Costal-2 (*Drosophila*) and Kif7 (vertebrates). Three new papers analysing mammalian Kif-7 function — one of them in a recent issue of *Current Biology* — now set the record straight on this matter [2–4].

The discovery of the *Drosophila costal-2* gene by Whittle [5] in 1973 was an almost miraculous event: the mutation had arisen simultaneously with a second site enhancer (named *Costal-1*) — a unique combination that yielded flies with striking pattern duplications in their wings [5] (Figure 1). At the time, the generation of similarly duplicated limbs by means of ZPA (zone of polarising activity) grafts in chick embryos (Figure 1) was a major preoccupation of developmental biologists, yet this first glimpse of the genetic pathway underlying such duplications went largely unnoticed for twenty years, only to resurface following the cloning of the *Sonic hedgehog* gene in 1993. This prompted a series of studies demonstrating how the ectopic expression of orthologous *Hh* genes could elicit pattern duplications in chick and *Drosophila* wings essentially identical to those described by Saunders [6] and Whittle [5], respectively, some two decades earlier.

Molecular cloning identified Costal-2 (Cos-2) as a divergent member of the kinesin family and subsequent studies highlighted its critical role in supporting kinase-mediated mechanisms that regulate the activity and action of Ci, the transcriptional effector of *Drosophila* Hh signalling [7]. Cos-2 physically interacts with Ci and several serine-threonine kinases, including protein kinase A (PKA) and Fused. The interaction enables PKA-mediated phosphorylation of Ci, priming Ci processing to a transcriptional repressor form in the absence of a Hh signal. In contrast, Fused promotes Hh target gene activation by preventing the action of Suppressor-of-fused (Su(fu)), a protein that binds to the activator form of Ci and inhibits its access to the nucleus. Thus, Cos-2 both represses Hh target genes when the Hh ligand is absent and promotes maximal levels of signalling in response to the ligand. The activity of *Drosophila* Cos-2 is governed, at least in part, by its interaction with the carboxy-terminal tail of Smo, a serpentine transmembrane protein essential for all Hedgehog signalling in both the fly and vertebrates. When, Hh ligand is absent, the Hh receptor Patched (Ptc) inhibits Smo. Upon Hh binding to Ptc, Smo accumulates at the membrane where a conformational change is thought to facilitate Cos-2 binding, thus modifying Cos-2-dependent control of Ci and microtubule-based movement of Cos-2–Ci complexes [8].

The core members of the Hh pathway — Patched, Smoothened, PKA and the Gli proteins (Ci homologues) — are conserved between *Drosophila* and vertebrates. It thus seemed unsurprising that in zebrafish, morpholino oligonucleotide mediated knockdown of Kif7, the vertebrate Cos2 orthologue, should cause ectopic pathway activity [9]. However, Varjosalo and colleagues [10] subsequently reported that Kif7 knock-down in mouse cell lines did not impact Hh signalling. Furthermore, they failed to observe any biochemical interaction of the cargo-domain of Kif7 with Gli-factors, or between Kif7 and the carboxy-terminal tail of Smo. These data pointed to an apparent divergence in the mechanism of Hh signal transduction between mammals and flies. Moreover, targeted mutation of a mouse orthologue of Fused, an obligate component of the pathway in *Drosophila* and a regulator of zebrafish Hedgehog signalling [11], showed no effect on Hh signalling in mice [12,13], whereas targeted mutation of Su(fu), a dispensable pathway component in *Drosophila*, caused a massive derepression of the mammalian Hh pathway [14]. Finally, whereas a function for the primary cilium is clear in mammals [1], the primary cilium plays no role in *Drosophila*, and its requirement for zebrafish Hh signalling is disputed [15,16], suggesting that the scaffold function of Cos2 in *Drosophila* and zebrafish may be subsumed by the primary cilium in mammalian cells.

Three new reports [2–4] examining independently generated Kif7 mutant alleles in the mouse — two null alleles and a missense mutation (L130P) within the motor domain of Kif7 — now show unequivocally that Kif7 does indeed play a central role in regulating mammalian Hh signalling, adding a cautionary note to the conclusions drawn from knock-down approaches using cultured cells. All three mutants exhibit broadly similar phenotypes, and the analysis predominantly focused on two well-studied aspects of the action of one Hh homolog, Sonic Hedgehog (Shh): control of digit formation in the limb and patterning

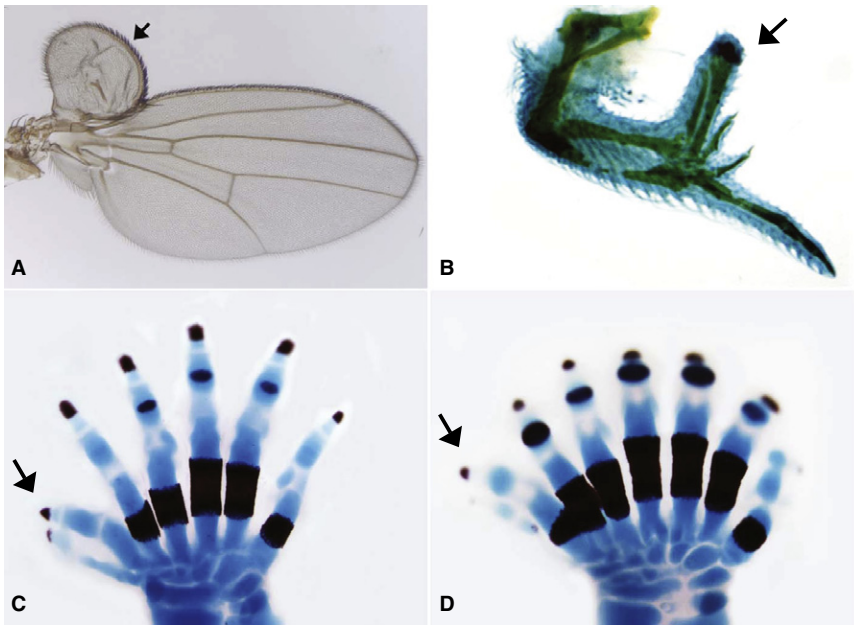

Figure 1. Pattern duplications caused by ectopic Hh signalling in flies and vertebrates.

Duplicated structures are indicated by arrows in all panels. (A) Loss of Cos-2 (here in the Cos1 background that facilitated its initial discovery) causes pattern duplications in the *Drosophila* wing. Reproduced with permission from [20]. (B) In chicken, a graft of the Hh-secreting ZPA causes a similar duplication in the wing (image courtesy of Cliff Tabin). Likewise in mouse, altered Hh signalling, by loss of Gli3 (C) and Kif7 activity (D), causes duplications in the limbs (images courtesy of C.C. Hui).

of the neural tube. In the vertebrate limb, Shh signalling antagonises proteasome-based processing of Gli3 an amino-terminal repressor form (Gli3-R) and thus controls the numbers of digits formed in the hand and foot plates [17]. Kif7 mutant mice display a striking preaxial polydactyly suggestive of ectopic derepression of Gli3 target genes in anterior regions of the limb where Shh does not normally act (Figure 1). In line with this observation, homozygous Kif7 mutant embryos show an increase in the ratio of full-length to repressor forms of Gli3. Thus, Kif7 is required to ensure normal levels of Gli3-repressor production.

In the neural tube, concentration-dependent Shh signalling originating from the ventrally located notochord and floorplate also attenuates Gli3 repressor levels, and this is sufficient for the specification of motor neuron progenitors. In Kif7 mutant embryos, the motor neuron progenitor population expands dorsally, again suggesting that Gli3 repressor levels are lowered independent of Shh signalling. The fate specification of more ventrally located pV3 interneurons and the ventral-most floor plate cell is dependent on Gli-activator forms, predominantly Gli2. In Kif7 mutants, pV3 cells expand dorsally — a phenotype enhanced by the genetic removal of Gli3 and suppressed by the reduction of Gli2 [3,4] — arguing that Kif7 also modulates Gli2 activity. Indeed, Gli2 levels rise in Kif7 mutants and transfection studies demonstrate that Kif7 inhibits Gli2-mediated activation of targets [2,3]. Taken together, these data support a model whereby Kif7 suppresses Gli-activator responses at least in part through controlling Gli2 levels. Kif7-based trafficking of Gli2 and Gli3 to the proteasome is one unifying model to reconcile the different effects of Kif7 on Gli-repressor and Gli-activator forms. Like Cos-2, Kif7 also promotes the response to Shh signalling. Induction of floorplate requires the highest level of Hh signalling, and the floorplate is reduced in Kif7 mutant embryos [2] — a phenotype dramatically enhanced by the removal of a single allele of Gli2 [3]. Furthermore, the ectopic induction of the floorplate upon removal of Ptch1-mediated inhibition of Smo is partially suppressed in Ptch1/Kif7 compound mutants [4].

Cell culture studies point to a physical interaction between Kif7 and Smo, Su(Fu) and the Gli proteins. Thus, Kif7 may act both as a scaffold to enable interactions that relay signalling from Smo to Gli proteins and as a motor to move these components within the cell [2,3]. All three mammalian Gli proteins and Su(Fu) can move to the tip of the primary cilium [18]. Interestingly, Kif7 localizes to the base of the primary cilium in the absence of Shh, but moves to the tip of the cilium when a Shh signal is present. Moreover, cilial trafficking of both Gli2 and Gli3, but not Su(Fu), is impaired in Kif7 mutants [2–4]. The observation that the proteasome also localises to the base of the cilium supports the notion of Kif7-facilitated processing of Gli2 and Gli3 in the absence of Shh. When a Shh signal is present, Kif7 may prevent Gli2 and Gli3 processing by promoting their accumulation within the primary cilium. Whereas reduced Gli-repressor levels are sufficient to derepress a subset of Gli-targets, others require the direct action of Gli-activator forms in the nucleus. Here, Su(Fu) presents a conundrum as Su(Fu) binds to, and strongly inhibits, Gli-A activity [2,3,15,19] but also localises to the tip of the primary cilium [2]. Intriguingly, normal Su(Fu)-mediated suppression of Gli-activator function appears to be largely independent of the primary cilium [19]. This suggests a model whereby effective trafficking of Gli-activator to the tip of the primary cilium, through a Kif7 microtubule-based motor-driven mechanism, may enable the uncoupling of Su(Fu) from Gli-activator, perhaps coincident with the loading of Gli-activator onto retrograde dynein-driven cargo complexes that move Gli-activator from the primary cilium to the nucleus.

Considering what we now know about Cos-2/Kif7 action in *Drosophila* and vertebrates, perhaps we should be more surprised that Cos-2 function is retained in *Drosophila* despite the demise of the primary cilium. The continuing requirement for Cos-2 in *Drosophila* may reflect a simple Cos2-based scaffolding function. However, the observation that Cos-2 has motor activity [8] suggests that the trafficking of Hh pathway components within the *Drosophila*

cell may have closer parallels to the dynamic action of vertebrate Kif7.

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Animal Cognition: Aesop's Fable Flies from Fiction to Fact

A new study shows that rooks are able to spontaneously drop stones into a tube of water to obtain a floating worm. This sophisticated problem solving raises intriguing questions about the use of imagination in animals.

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Do Aesop's fables (Figure 1) reflect the behaviour of real animals? While talking foxes and racing tortoises may be unlikely, Bird and Emery's recent findings suggest there may be a kernel of truth to one of Aesop's most famous stories. In the fable of the crow and the pitcher, a clever crow drops stones into a jug of water in order to raise the level and ease its thirst (Figure 1) [1]. The rooks in the experiment reported recently in *Current Biology* by Bird and Emery [2] did something strikingly similar — they dropped stones into a tube of water in order to bring a floating worm within reach. Two of the four rooks tested were able to spontaneously solve this problem on the first trial. The rooks were then able to rapidly learn to drop big stones, rather than small ones, into the tube. They also rapidly learnt to drop stones only into tubes containing water, and not those containing sawdust.

At first glance, the way the birds solved these tasks seems remarkably insightful and human-like. The rooks only put in sufficient stones to bring the worm within reach, and then did not continue to add stones once the worm had been removed. The rooks also appeared to examine the problem before putting stones into the tube, consistent with the idea that they initially assessed the task. One interpretation of these results is that the rooks had immediate causal knowledge of the task [3]. That is, they understood how the stones would interact with the water and therefore could estimate how high the water would rise once a certain number of stones were put into the tube. As the fable tells it, the crow put the stones into the pitcher because it *knew* that this would cause the water level to rise.

However, the follow-up experiments preclude the use of such human-like causal knowledge. If the rooks had understood how stones interact with water they should have also known that bigger stones would displace more water. In contrast to this expectation, the rooks did not immediately use large stones when presented with a choice between small and large stones, although they quickly learnt to do so. The rooks also did not seem to have knowledge of the peculiar causal